DNA 3130 SPECTRAL CALIBRATION USING DS-33 (YFILER)

A. SCOPE

A spectral calibration creates a matrix that corrects for the overlapping fluorescence emission spectra of the dyes. Although each of these dyes emits its maximum fluorescence at a different wavelength, there is some overlap in the emission spectra between the dyes. The goal of multicomponent analysis is to effectively correct for spectral overlap. Performing a spectral calibration is similar to performing a sample run, except that calibration standards are run in place of samples and a spectral calibration module is used in place of a run module.

B. QUALITY CONTROL

B.1 See DOC ID 1835 to determine reagent expiration dates.

B.2 Do not clean any components or accessories of the 3130 with bleach or ethanol. Clean with deionized water.

B.3 Hi-Di Formamide: To prevent repeated thaw and re-freezing of formamide, aliquot formamide into approximately 500 and 1000 µL volumes after initially thawing the 25 mL bottle. Appropriately discard any unused aliquot of thawed formamide.

C. SAFETY

C.1 Hi-Di Formamide: exposure causes eye, skin, and respiratory tract irritation. It is a possible developmental and birth defect hazard.

C.2 All appropriate SDS sheets must be read prior to performing this procedure.

C.3 Protective gloves, a lab coat and eye protection must be worn at all times when performing this procedure.

C.4 Distinguish all waste as general, biohazard, or sharps and discard appropriately.

D. REAGENTS, STANDARDS, AND CONTROLS

D.1 3130 Performance Optimized Polymer (POP-4 polymer)

D.2 Matrix Standard Set DS-33 for Yfiler which includes the following dyes: 6FAM, VIC, NED, PET, and LIZ

D.3 AB Prism 10X Genetic Analyzer Buffer w/ EDTA to make a 1X working buffer:

Add 25 mL of Buffer 10X to 225 mL of DI H_2O to make 250 mL of working buffer, or make up a 1000 mL of the working buffer by adding 100 mL of Buffer 10X (4 bottles) to 900 mL DI H_2O.

D.4 Hi-Di Formamide

D.5 DI H_20

E. EQUIPMENT & SUPPLIES

E.1 Equipment

E.1.1 AB 3130 Genetic Analyzer (instrument, computer and appropriate software)
E.1.2 AB 36cm capillary array
E.1.3 AB Prism Genetic Analyzer sample septa and plates
E.1.4 Thermal cycler
E.1.5 Pipettes
E.1.6 Vortexer
E.1.7 Frozen plate block
E.1.8 96-well plate retainer and base
E.1.9 96-well plate centrifuge

E.2 Supplies

E.2.1 3130 Genetic Analyzer buffer vials/reservoirs/reservoir septa
E.2.2 Pipette tips
E.2.3 Microcentrifuge tubes
E.2.4 Scalpel

F. PROCEDURES

NOTE: A spectral calibration using DS-36 (GlobalFiler, DOC ID 12669) and DS-33 (YFiler) may be prepared and run on the same plate.

F.1 Combine 5 µl of DS-33 spectral standard (includes 6-FAM, VIC, NED, PET and LIZ dyes) with 195 µl of Formamide. Vortex thoroughly.

Note: Depending on the sensitivity of the 3130 instrument, it may be necessary to adjust the concentration of the dyes in the dyes / Hi-Di formamide mixture.

F.2 Dispense 10 µl of the spectral / formamide mixture into a 96 well plate. Dispense into 4 wells, (one per capillary).

F.3 Cover the plate and denature for 3 minutes at 95°C, immediately place in the frozen plate holder for 3 minutes.

F.4 Centrifuge the plate, use a retainer clip to secure it onto the plate base, and place this on a 3130 instrument for a run.

F.5 In **Plate Manager** select, **new**, name the run spectral mmddyy.

F.6 Under application select **Spectral Calibration.**

F.7 Select the plate default value of **96 well**. Add operator initials, select **OK.**

F.8 Fill in the respective plate locations of the spectral standard e.g. A01-D01 with the sample name DS33.

F.9 Select instrument protocol as **G5_Spectral**. An additional run may be inserted for a second injection.

F.10 Go to **Run Scheduler** select **find all,** highlight the plate document created in plate manager and link the plate document to the yellow plate by mouse clicking on the plate diagram. Select the green arrow to run.

G. INTERPRETATION GUIDELINES

G.1 Upon completion review the pass or fail status of each capillary in the **Instrument status / Events Messages**. In a good quality calibration each capillary should have a Q-value of above 0.95 and a condition number within the range of 7 to 12.

G.2 If the entire spectral failed go to the trouble shooting flow chart in the 3130 documents on the desktop.

G.3 Go to **Spectral Viewer**, ensure the Dye set is G5, and select a well position that had spectral standards in it from the plate layout diagram. A dark green box in the sample position indicates pass and a brown box indicates failed.

G.4 For the selected capillary, verify that the order of the peaks in the spectral profile (pixel vs. signal intensity) from left to right are blue, green, yellow, red, and orange.

G.5 Verify that the peaks in the spectral profile do not contain gross overlaps, dips, or other irregularities.

G.6 If acceptable click **rename** and rename the spectral run with the date of the run.

G.7 Click **OK**

H. REFERENCES

H.1 Applied Biosystems 3130/3130xl Genetic Analyzers Using Data Collection Software v3.0, February 8, 2005.

H.2 Applied Biosystems 3130/3130xl Genetic Analyzers Getting Started Guide November 2004.